Title: Perfect score:

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AAW21816
     18
     313
63.0
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     12
                                                                                                                                                                                                                                                                                                                                      August 21, 2002, 10:15:23 ; Search time 30.33 Seconds (without alignments) 1102.315 Million cell updates/sec
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| SIDSI/gcgdata/hold-geneseq/geneseqp-embl/AA1980.DAT:*
| SIDSI/gcgdata/hold-geneseq/geneseqp-embl/AA1981.DAT:*
| SIDSI/gcgdata/hold-geneseq-geneseqp-embl/AA1981.DAT:*
| SIDSI/gcgdata/hold-geneseq-geneseqp-embl/AA1991.DAT:*
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| SIDSI/gcgdata/hold-geneseqy-embl/AA1991.DAT:*
| SIDSI/gcgdata/hold-geneseqy-geneseqp-embl/AA1991.DAT:*
| SIDSI/gcgdata/hold-geneseqy-geneseqp-embl/AA1999.DAT:*
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                                                         GenCore version 4.5 Copyright (c) 1993 - 2000 Compugen Ltd
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Maximum Match 100%
Listing first 45 summaries
                                                                                                                                                                                                                                                   OM protein - protein search, using sw model
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Gapop 10.0 , Gapext 0.5
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Maximum DB seq length: 200000000
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Pred. No. is the number of results predicted by chance to have a score greater than or equal to the score of the result being printed, and is derived by analysis of the total score distribution.

SUMMARIES

Description	Atlantic cod heat-	Atlantic cod heat-	Human colon cancer	Uracil DNA glycosy	Mutant uracil DNA	Mutant uracil DNA	Mutant uracil DNA	Mutant uracil DNA	Cytosine DNA glyco	Mutant uracil DNA	Mutant uracil DNA
ID		AAU04940	AAG74939	AAW21814	AAW21823	AAW21822	AAW21820	AAW21821	AAW21815	AAW21824	AAW21819
DB	22	22	22	18	18	18	18	18	18	18	18
% Query e Match Length DB I	301	301	292	313	313	313	313	313	313	313	313
% Query Match	9.66	98.8	64.3	63.5	63.3	63.3	63.3	63.2	63.2	63.2	63.1
Score	1596	1421	1030.5	1018.5	1015.5	1014.5	1014.5	1013.5	1013.5	1012.5	1011.5
Result No.	1	7	3	4	'n	9	7	80	6	10	11

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Sequence
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     Atlantic cod; heat-labile uracil-DNA glycolsylase; UNG; UDG;
PCR control; LCR control; ligase chain reaction; carry-over prevention.
                                                                 The sequence is an Atlantic cod heat-labile uracil-DNA glycosylase, (UNG/UDG). The enzyme is useful in monitoring and/or controlling a reaction system multiplying DNA sequences, e.g. PCR (polymerase chain reaction) or LCR (ligase chain reaction). The enzyme is also useful in carry-over prevention procedures.
                                                                                                                                                                                                                                                                                                                                                             61 RMAKNKKAALDKIRAKATPAGFGETWRRELAAEFEKPYFKQLMSFVADERSRHTVYPPAD 120
                                                                                                                                                                                                                                                                                 121 QVYSSTEMCDIQDVKVVILGQDPYHGPNQAHGLCFSVQKPVPPPPPSLVNIYKELCTDIDG 180
                                                                                                                                                                                                                                                                                                                      181 FKHPGHGDLSGWAKQGVLLLNAVLTVRAHQANSHKDRGWETFTDAVIKWLSVNREGVVFL 240
                                                                                                                                                                                 Gaps
                                                                                                                                                                                                             or
                                                                                                                                                                                                   1 MLFKLGLCQRCISSNRVLPGLLIPQTLCFSKLMKITPKKLRSSNVEQKTSSPQLSVEQLE 60
         New cod liver uracil-DNA glycosylase enzyme, useful in monitoring controlling a reaction system multiplying DNA sequences or in
                                                                                                                                                                                                                                                                                            ;
                                                                                                                                                           Length 301;
                                                                                                                                                                               Indels
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   heat-labile uracil-DNA glycosylase, UNG #2.
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                                                                                                                                                           DB 22;
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 Gjellesvik
                                                                                                                                                          Score 1596; DB 2:
Pred. No. 9e-156;
                                                                                                                                                                               0; Mismatches
                                                                                                                                                                                                                                                                                                                                                                                                                                                                       AAU04940 standard; Protein; 301 AA.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 Guddal PH,
                                               2; Page 52-54; 59pp; English
                             carry-over prevention procedures
                                                                                                                                                         99.68;
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             10-JAN-2001; 2001WO-NO00008
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 12-JAN-2000; 2000NO-0000163
27-OCT-2000; 2000NO-0005428
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               (first entry)
                                                                                                                                                                              Matches 300; Conservative
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 Willasen NP,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              (BIOT-) BIOTEC ASA
                                                                                                                                                                     Best Local Similarity
                                                                                                                              301 AA;
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                                                                                                                              Sequence
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The sequence is an Atlantic cod heat-labile uracil-DNA glycosylase, (UNG/UDG). The enzyme is useful in monitoring and/or controlling a reaction system multiplying DNA sequences, e.g. PCR (polymerase chain reaction) or LCR (ligase chain reaction). The enzyme is also useful in carry-over prevention procedures.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      SHKDRGWETFTDAVIKWLSVNREGVVFLLWGSYAHKKGATIDRKRHHVLQAVHPSPLSAH 272
                                                               New cod liver uracil-DNA glycosylase enzyme, useful in monitoring or controlling a reaction system multiplying DNA sequences or in
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       33 MKITPKKLRSSNVEQKTSSPQLSVEQLERMAKNKKAALDKIRAKATPAGFGETWRRELAA
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  LCFSVQKPVPPPPSLVNIYKELCTDIDGFKHPGHGDLSGWAKQGVLLLNAVLTVRAHQAN
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   Human; colon cancer; colon cancer antigen; diagnosis; detection; colorectal carcinoma; chromosome 12.
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                                                                                                                                                                                                                                                                                                                                                                                                        Length 301;
                                                                                                                                                                                                                                                                                                                                                                                                                                                       Indels
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                                                                                                                                                                                                                                                                                                                                                                                                      Score 1421; DB 22;
Pred. No. 9.4e-138;
2; Mismatches 1;
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                                                                                                                                                    Claim 2; Page 54-56; 59pp; English
                                                                                                            carry-over prevention procedures
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         AAG74939 standard; Protein; 292
                                                                                                                                                                                                                                                                                                                                                                                                      Query Match 88.6%;
Best Local Similarity 98.9%;
Matches 266; Conservative
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           (HUMA-) HUMAN GENOME SCI INC
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       99US-0157137.
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2001-451854/48.
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                  N-PSDB; AAS09499
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03-NOV-1999;
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us-09-758-017a-2.rag

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313 AA;
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                                                   09-JAN-1997;
 WO9725416-A2
                                                                            09-JAN-1996;
                           17-JUL-1997.
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                                                                    AAH32943 to AAH37195 and AAG73514 to AAG77788 represent human colon cancer-associated nucleic acid molecules (N) and proteins (P), where the proteins are collectively known as colon cancer antigens. The colon cancer antigens. The colon cancer antigens have cytostatic acitivity and can be used in gene therapy and vaccine production. N and P may be used in the prevention, cancer antigens and treatment of diseases associated with inappropriate P expression. For example, N and P may be used to treat disorders associated with decreased expression by rectifying mutations or deletions in a patient's genome that affect the activity of P by expressing inactive proteins or to supplement the patients own production of P. Additionally, N may be used to produce the colon cancer-associated Ps. by inserting the nucleic acids into a host cell and culturing the cell context of colorectal carcinomas and cancers. AAH37196 to AAH37204 and AAB77789 represent sequences used in the exemplification of the present invention.

N.B. Pages 666 to 682 and page 7053 of the sequence listing were missing at time of publication, meaning no sequences are present for SEQ ID NO:1027 to 1052, 7921 and 7922.
                                                                                                                                                                                                                                                                                                                                                                                                      5;
            Nucleic acids encoding 4277 human colon cancer-associated polypeptides, useful for preventing, diagnosing and/or treating colorectal cancers -
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            85 TWRRELAAEFEKPYFKQLMSFVADERSRHTVYPPADQVYSSTEMCDIQDVKVVILGQDPY 144
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  HGPNQAHGLCFSVQKPVPPPPSLVNIYKELCTDIDGFKHPGHGDLSGWAKQGVLLLNAVL 204
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                                                                                                                                                                                                                                                                                                                                                                                                                              84
                                                                                                                                                                                                                                                                                                                                                                                                                                         3; Gaps
                                                                                                                                                                                                                                                                                                                                                                                                                           27 LCFSKLMKITPKKLRSSNVEQKT -- SSPQLSVEQLERMAKNKKAALDKIRAKATPAGFGE
                                                                                                                                                                                                                                                                                                                                                                           DB 22; Length 292;
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    Uracil DNA glycosylase; UNG2; mutagenesis; DNA sequencing;
DNA modification; cell killing.
                                                                                                                                                                                                                                                                                                                                                                                                   52; Indels
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/label= Nuclear_localisation_peptide
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                                                                                                                                                                                                                                                                                                                                                                                       .5e-97
                                                                                                                                                                                                                                                                                                                                                                         Query Match 64.3%; Score 1030.5; Best Local Similarity 69.0%; Pred. No. 1.5e Matches 191; Conservative 31; Mismatches
                                               Claim 11; Page 7239-7240; 9803pp; English.
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213 SHKDRGWETFTDAVIKWLSVNREGVVFLLWGSYAHKKGATIDRKRHHVLQAVHPSPLSAH 272
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            A new form of uracil DNA glycosylase (AAW21814), designated UNG2, is capable of releasing uracil bases from single and/or double stranded DNA. Its amino acid sequence was deduced from a cDNA clone (AAT73564) obtd. from a human NT2 neuronal precursor cell library. UNG2 represents an alternatively spliced form of UNG that includes a 44-amino acid presequence (see also AAW21818) that is not essential for catalytic activity. UNG2 can be mutated to provide cytosine or thymine DNA glycosylases (see also AAW21815-16). Recombinant DNA glycosylases can be expressed in host cells for use modification and in cell killing.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              Gaps
                                                                                                                                                                                                                                                                                                                                                                      DNA glycosylase capable of releasing cytosine, thymine or uracil
bases from DNA - useful in in vitro and/or vivo mutagenesis systems
to remove contaminating DNA prior to PCR amplification
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         35 ITPKKLRSSNVEQKT--SSPQLSVEQLERMAKNKKAALDKIRAKATPAGFGETWRRELAA
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             63.5%; Score 1018.5; DB 18; Length 313; 69.9%; Pred. No. 2.8e-96; Live 31; Mismatches 47; Indels 3;
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        47; Indels
                                                                                                                                                                                                                   Tainer JA;
                                                                                                                                                                                                                   Slupphaug G,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  Uracil DNA glycosylase; UNG2; mutagenesis.
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285 rgffgcrhfsktnellqksgkkpidwkel 313
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           Claim 1; Page 47-48; 60pp; English.
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                                                                                                                                                                                                                   Mol CD,
                                                          96GB-0000384
97WO-GB00057
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Best Local Similarity 69.98,
Matches 188; Conservative
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                                                                                                                     (DZIE/) DZIEGLEWSKA H E. (NYFO-) NYFOTEK AS.
                                                                                                                                                                                                                Kavli B, Krokan HE,
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(first entry)

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28-SEP-1997
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           DNA glycosylase capable of releasing cytosine, thymine or uracil bases from DNA - useful in in vitro and/or vivo mutagenesis systems to remove contaminating DNA prior to PCR amplification
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                                                                                                                                                /note= "site of Ser-178 to Ala substn."
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    Tainer JA;
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   63.3%; Score 1015.5; DB 69.5%; Pred. No. 5.7e-96; iive 32; Mismatches 47
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           Example 3; Refer to Page 47-48; 60pp; English.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    Slupphaug G,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        ||| ||:|||| | ||: || :||:|| | rdffgcrhfsktnellqksgkkpidwkel 313
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    RGFLGCKHFSKANGLLKLSGTEPINWRAL 301
                                                                                        Location/Qualifiers
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    AAW21822 standard; Protein; 313 AA
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                                                                                                                                                                                                                                                                                                                        97WO-GB00057
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                                                                                                                     Misc-difference
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                                      Synthetic.
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Mutant DNA glycosylases (AAM21819-256) were produced by site-directed mutagenesis of human uracil DNA glycosylase UNG2 cDNA (see also AAT73564) and expression in Escherichia coli. None of these mutants showed cytosine DNA glycosylase or thymine DNA glycosylase activity. In contrast, an M204D substn. (see also AAW21815) provided thymine DNA glycosylase activity, and a Y147A substn. (see also AAW21816) provided thymine DNA glycosylase activity, and a results demonstrated the significance of Asn204 for specific binding of uracil-containing DNA and the significance of the Tyr147 side chain ring structure for preventing binding of thymine.
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        Length 313;
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                                                                                                                                                                                      /note= "site of Tyr-147 to Phe substn."
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             JA;
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 .5; DB 18;
7.2e-96;
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             Tainer
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      63.3%; Score 1014.5;
ilarity 69.5%; Pred. No. 7.2e
Conservative 32; Mismatches
                                                 mutagenesis
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rgffgcrhfsktnellqksgkkpidwkel 313
Mutant uracil DNA glycosylase (Y147F).
                                                                                                                                    Location/Qualifiers
156
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                                              DNA glycosylase; UNG2;
                                                                                                                                                                                                                                                                                                                                  97WO-GB00057.
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nes 187; Conserv
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                                                                                                                                                                  Misc-difference
                                                                                                                                                                                                                                       WO9725416-A2
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                                                                                                Synthetic
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           EFEKPYFKQLMSFVADERSRHTVYPPADQVYSSTEMCDIQDVKVVILGQDPYHGPNQAHG 152
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      DNA glycosylase capable of releasing cytosine, thymine or uracillabases from DNA - useful in in vitro and/or vivo mutagenesis systems to remove contaminating DNA prior to PCR amplification
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 47; Indels
                                                                                                                                                                                                       /note= "site of Asp-145 to Glu substn."
                                                                                                                                                                                                                                                                                                                                                                                                 Tainer JA;
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 63.3%; Score 1014.5; DB 69.5%; Pred. No. 7.2e-96; ive 32; Mismatches 47
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                Example 3; Refer to Page 47-48; 60pp; English.
                                                                                                                                                                                                                                                                                                                                                                                                 Slupphaug G,
                                                                                                                  Uracil DNA glycosylase; UNG2; mutagenesis.
                                                                                      Mutant uracil DNA glycosylase (D145E)
                                                                                                                                                                       ney Location/Qualifiers
Misc-difference 154
    ¥.
AAW21820 standard; Protein; 313
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                                                                                                                                                                                                                                                                                            97WO-GB00057
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Matches 187; Conservative
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                                                                                                                                               Synthetic
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                              AAW21820;
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                                                                                                                                                                                                                                                                                                                                                                    (NYFO-)
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Mutant DNA glycosylases (AAW21819-256) were produced by site-directed
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    mutagenesis of human uracil DNA glycosylase UNG2 CDNA (see also AAT73564) and expression in Escherichia coli. None of these mutants showed cytosine DNA glycosylase ortivity. In contrast, an N204D substn. (see also AAM21815) provided cytokine DNA glycosylase activity, and a Y147A substn. (see also AAM21815) provided thymine DNA glycosylase activity. The results demonstrated the significance of Asn204 for specific binding of uracil-containing DNA and the significance of the Tyr147 side chain ring structure for preventing binding of thymine.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          93 EFEKPYFKQLMSFVADERSRHTVYPPADQVYSSTEMCDIQDVKVVILGQDPYHGPNQAHG 152
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  Gaps
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          DNA glycosylase capable of releasing cytosine, thymine or uracil bases from DNA - useful in in vitro and/or vivo mutagenesis systems to remove contaminating DNA prior to PCR amplification
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                                                                                                                                                                                                                                                                        /note= "site of Asp-145 to Asn substn."
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                                                                                                                                                                                                                                                                                                                                                                                                                                                       Slupphaug G,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              32; Mismatches
                                                                                                                                                                                         Uracil DNA glycosylase; UNG2; mutagenesis.
Mutant uracil DNA glycosylase (D145N).
                                                                                                                                                                                                                                              Location/Qualifiers
                                                                                  AA.
                                                                                  Protein; 313
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                                                                                                                                                                                                                                                                                                                                                                                                              (DZIE/) DZIEGLEWSKA H E. (NYFO-) NYFOTEK AS.
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                WPI; 1997-372857/34.
                                                                                                                                                                                                                                                          Misc-difference 154
                                                                                AAW21821 standard;
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                                                                                                           AAW21821;
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Best Local S
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RGFLGCKHFSKANGLLKLSGTEPINWRAL 301

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105 efgkpyfiklmgfvaeerkhytvyppphqvftwtqmcdikdvkvvilgqdpyhgpnqahg 164
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     bases from DNA - useful in in vitro and/or vivo mutagenesis systems to remove contaminating DNA prior to PCR amplification
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   A novel cytosine DNA glycosylase (CDG) (AAW21815) is capable of releasing both cytosine and uracil bases from single and/or double stranded DNA. It is obtd. by site-directed mutagenesis of human uracil DNA glycosylase UNG2 cDNA (see also AAT73564) such that the encoded polypeptide has a Asn-204 to Asp amino acid substn. that results in gain of CDG activity. Recombinant enzyme can be expressed in transformed host cells for use in mutagenesis (in vivo or in vitro) systems, to remove contaminating DNA prior to PCR, in DNA modification, in cell killing, and in DNA sequencing to determine the position of cytokine bases.
                          DNA glycosylase capable of releasing cytosine, thymine or uracil
bases from DNA - useful in in vitro and/or vivo mutagenesis sys
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                                                                                                                                                                                                                                                                                                                                                                                                glycosylase; uracil DNA glycosylase; UNG2;
DNA sequencing; DNA modification; cell killing.
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                                                                                                        285 rgffgcrhfsktnellqksgkkpidwkel 313
                                                                                    RGFLGCKHFSKANGLLKLSGTEPINWRAL 301
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                                                                                                                                                                                                                                 AAW21815 standard; Protein; 313
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69.5%;
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                                                                                                                                                                                                                                                                                                                                                           Cytosine DNA glycosylase.
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Best Local Si
Matches 187;
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Mutant DNA glycosylases (AAW21819-256) were produced by site-directed mutagenesis of human uracil DNA glycosylase UNG2 cDNA (see also AAR73564) and expression in Escherichia coli. None of these mutants showed cytosine DNA glycosylase or thymine DNA glycosylase activity. In contrast, an N204D substn. (see also AAW21815) provided thymine DNA glycosylase activity, and a Y147A substn. (see also AAW21816) provided thymine DNA glycosylase activity. The results demonstrated the significance of Asn204 for specific binding of uracil-containing DNA and the significance of the Tyr147 side chain ring structure for preventing binding of thymine.
                                                                                                                272
                                                                                                                                              Gaps
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                          SHKDRGWETFTDAVIKWLSVNREGVVFLLWGSYAHKKGATIDRKRHHVLQAVHPSPLSAH
LCFSVQKPVPPPPSLVNIYKELCTDIDGFKHPGHGDLSGWAKQGVLLLNAVLTVRAHQAN
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.larity 69.5%; Pred. No. 1.2e-95;
Conservative 31; Mismatches 48; In
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                                                                                                                                                                                                                                 RGFLGCKHFSKANGLLKLSGTEPINWRAL 301
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Misc-difference 213
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Query Match
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                                        152
LCFSVQKPVPPPPSLVNIYKELCTDIDGFKHPGHGDLSGWAKQGVLLLNAVLTVRAHQAN 212
                                                                                                       SHKDRGWETFTDAVIKWLSVNREGVVFLLWGSYAHKKGATIDRKRHHVLQAVHPSPLSAH 272
                                                                                                                                                        DNA glycosylase capable of releasing cytosine, thymine or uracil bases from DNA - useful in in vitro and/or vivo mutagenesis systems to remove contaminating DNA prior to PCR amplification
                                     93 EFEKPYFKQLMSFVADERSRHTVYPPADQVYSSTEMCDIQDVKVVILGQDPYHGPNQAHG
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                                                                                                                                                                                                          ||| ||:|||| | ||:||: || ||:||: || 285 rgffgcrhfsktnellqksgkkpidwkel 313
                                                                                                                                                                                           RGFLGCKHFSKANGLLKLSGTEPINWRAL 301
                                                                                                                                                                                                                                                                                                                                                                 Mutant uracil DNA glycosylase (Q144L).
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                                                                                                                                                                                                                                                                                                                                                                                                                  Synthetic
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           (NYFO-)
                                                                                        153
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152
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                                                                      284
                            Gaps
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    Thymine DNA glycosylase; uracil DNA glycosylase; UNG2; mutagenesis; DNA sequencing; DNA modification; cell killing.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 A novel thymine DNA glycosylase (TDG) (AAW21816) is capable of releasing both thymine and uracil bases from single and/or double stranded DNA. It is obtd. by site-directed mutagenesis of human uracil DNA glycosylase UNG2 cDNA (see also AAT/3564) such that the encoded polypeptide has a Tyr-147 to Ala amino acid substn. that results in gain of TDG activity. Recombinant enzyme can be expressed in transformed host cells for use in mutagenesis (in vivo or in vitro) systems, to remove contaminating DNA prior to
                                                                                                        93 EFEKPYFKQLMSFVADERSRHTVYPPADQVYSSTEMCDIQDVKVVILGQDPYHGPNQAHG
                                                                                                                           35 ITPKKLRSSNVEQKT--SSPQLSVEQLERMAKNKKAALDKIRAKATPAGFGETWRRELAA
                                                                                                                                                                 153 LCFSVQKPVPPPPSLVNIYKELCTDIDGFKHPGHGDLSGWAKQGVLLLNAVLTVRAHQAN
                                                                                                                                                                                213 SHKDRGWETFTDAVIKWLSVNREGVVFLLWGSYAHKKGATIDRKRHHVLQAVHPSPLSAH
                           3;
                           48; Indels
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Score 1011.5; DB Pred. No. 1.5e-95
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 Slupphaug G,
                           31; Mismatches
                                                                                                                                                                                                                                                                                           273 RGFLGCKHFSKANGLLKLSGTEPINWRAL 301
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       Location/Qualifiers
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                                                                                                                                                                                                                                                                                                                                                                           Protein; 313
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63.1%;
69.5%;
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                                                                                                                                                                                                                                                                                                                                                                                                                                (first entry)
                           Matches 187; Conservative
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E
                                                                                                                                                                                                                                                                                                                                                                                                                                                          Thymine DNA glycosylase.
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                                                                                                                                                                                                                                                                                                                                                                          AAW21816 standard;
              Similarity
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Misc-difference
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   09-JAN-1997;
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               Synthetic.
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  Thymine
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showed cytosine DNA glycosylase or thymine DNA glycosylase activity. In contrast, an RV304D substn. (see also AAW21815) provided cytokine DNA glycosylase activity, and a Y147A substn. (see also AAW21816) provided thymine DNA glycosylase activity. The results demonstrated the significance of Asm204 for specific binding of uracil-containing DNA and the significance of the Tyr147 side chain ring structure for preventing binding of thymine.
                                                                                                                     62.9%;
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2000US-0650855.
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                                                                                                                                                                                                                                                                                                                                                                                                                                                            (first entry)
                                                                                                                                             Conservative
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                                                                                                                      Query Match
Best Local Similarity
Matches 187; Conserv
                                                                                                                                                                                                                                                                                                                                                                                                                AAU69754 standard;
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    Mc Cutchen-maloney
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          Escherichia coli.
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29-AUG-2000;
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                                                                                         Sequence
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                                                                                                                                                    EFEKPYFKQLMSFVADERSRHTVYPPADQVYSSTEMCDIQDVKVVILGQDPYHGPNQAHG 152
                                                                                                                                                                LCFSVQKPVPPPPSLVNIYKELCTDIDGFKHPGHGDLSGWAKQGVLLLNAVLTVRAHQAN 212
                                                                                                                                                                                                        SHKDRGWETFTDAVIKWLSVNREGVVFLLWGSYAHKKGATIDRKRHHVLQAVHPSPLSAH 272
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               Mutant DNA glycosylases (AAW21819-256) were produced by site-directed mutagenesis of human uracil DNA glycosylase UNG2 cDNA (see also AAT73564) and expression in Escherichia coli. None of these mutants
                                                                                                                                                                                                                                                   Gaps
                                                                                                         ITPKKLRSSNVEQKT--SSPQLSVEQLERMAKNKKAALDKIRAKATPAGFGETWRRELAA 92
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  cytosine, thymine or uracil
and/or vivo mutagenesis systems
PCR amplification
PCR, in DNA modification, in cell killing, and in DNA sequencing to determine the position of cytokine bases.
                                                                  Length 313;
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                                                                                      48; Indels
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              /note= "site of His-277 to Leu substn."
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          Tainer
                                                                 DB 18;
                                                              ;; Score 1009.5; DB
;; Pred. No. 2.3e-95;
31; Mismatches 48
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            Example 3; Refer to Page 47-48; 60pp; English.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        Slupphaug G,
                                                                                                                                                                                                                                                                                                                                                                                                                                               Uracil DNA glycosylase; UNG2; mutagenesis
                                                                                                                                                                                                                                                                                             ||| ||:|||| | ||: || :||:|
rgffgcrhfsktnellqksgkkpidwkel 313
                                                                                                                                                                                                                                                                                RGFLGCKHFSKANGLLKLSGTEPINWRAL 301
                                                                                                                                                                                                                                                                                                                                                                                                                         Mutant uracil DNA glycosylase (H268L).
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 DNA glycosylase capable of releasing
bases from DNA - useful in in vitro
to remove contaminating DNA prior to
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                                                                                                                                                                                                                                                                                                                                                          AAW21825 standard; Protein; 313
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                                                                63.0%;
69.5%;
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                                                                                   Matches 187; Conservative
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NYFOTEK AS.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       B, Krokan HE,
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                                                                          Similarity
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                                                              Query Match
Best Local (
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                                Sequence
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         Recombinant chimeric protein, useful for detecting and quantifying DNA
                                                                                                                                        35 ITPKKLRSSNVEQKT--SSPQLSVEQLERMAKNKKAALDKIRAKATPAGFGETWRRELAA 92
                                                                                                                                                                                                                                                   LCFSVQKPVPPPPSLVNIYKELCTDIDGFKHPGHGDLSGWAKQGVLLLNAVLTVRAHQAN
                                                                                                                                                                                                                                                                                                                                                           EFEKPYFKQLMSFVADERSRHTVYPPADQVYSSTEMCDIQDVKVVILGQDPYHGPNQAHG
                                                                                                                                                                                                                                                                                                                                                                                                                                            SHKDRGWETFTDAVIKWLSVNREGVVFLLWGSYAHKKGATIDRKRHHVLQAVHPSPLSAH
Length 313;
                                                      3;
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                                                      48; Indels
Score 1007.5; DB 18;
Pred. No. 3.8e-95;
1; Mismatches 48; I
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    285 rqffqcrhfsktnellqksqkkpidwkel 313
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2001-147511/15
               N-PSDB; AAF94351
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                                                            Sequences AAU69737-AAU69760 represent proteins which can be used in the synthesis of chimeric proteins comprising a DNA mutation-binding protein, a linker and a nuclease, by recombinant technology. The chimeric proteins care useful for detection, quantification and mapping of DNA sequence variations including mutations, for example, caused by damage and mismatches. The proteins are able to bind to the site of the DNA mutation and cut it out of the molecule. This is useful for early diagnosis of cancer and other diseases. The proteins used in the invention include human XPF (or ERC4), human xeroderma pigmentosum complementation groups A, C and E (XPA, XPC and XPE), human Muts homologue 2 (hMSH2), Serratia marcescens nuclease (Nucl), Thermus thermophilus Mut6, Escherichia coli Fapy-DNA glycosylase (Fpg), uracil DNA glycosylase (Mut1), synthetic T4 endonuclease V (T4 endo V), thymine DNA-glycosylase (TDG), E. coli UVT A, B and C, and E. coli
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                                                                                                                                                                                                                                                                                                                                                                              85 TWRRELAAEFEKPYFKQLMSFVADER-SRHTVYPPADQVYSSTEMCDIQDVKVVILGQDP 143
                                                                                                                                                                                                                                                                                                                                                                                                                                                 LTVRAHQANSHKDRGWETFTDAVIKWLSVNREGVVFLLWGSYAHKKGATIDRKRHHVLQA 263
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     144 YHGPNQAHGLCFSVQKPVPPPPSLVNIYKELCTDIDGFKHPGHGDLSGWAKQGVLLLNAV 203
                                                                                                                                                                                                                                                                                                                                                     1; Gaps
                                                                                                                                                                                                                                                                                                                                                                                             Haemophilus influenzae; essential bacterial gene; identification; otitis media; meningitis; upper respiratory tract infection; infection; antimicrobial.
mutations, e.g. in disease diagnosis, comprises mutation-binding
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    Haemophilus influenzae essential bacterial protein SEQ ID NO:14
                                                                                                                                                                                                                                                                                                                          DB 22; Length 229;
                                                                                                                                                                                                                                                                                                                         42.5%; Score 681.5; DB 22; Length 60.9%; Pred. No. 9e-62; ive 24; Mismatches 59; Indels
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        Disclosure; Page 96-97; 128pp; English.
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Matches 131; Conservative
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    Haemophilus influenzae
               protein and nuclease
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        (ABBO ) ABBOTT LAB.
                                                                                                                                                                                                                                                                                 229 AA;
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                                                                                                                                                                                                                                                                                    Sequence
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                                                                                                                                                                 AAF94345 to AAF94409 represent essential bacterial genes from Haemophilus influenzae, which encode the proteins given in AAB88492 to AAB88556. The present invention also describes methods for identifying essential bacterial genes (i.e. those essential to the survival of a bacterium) using a transposition system. The methods are used to identify essential genes from bacteria, especially H. Influenzae (which eauses otitis media, meningitis and upper respiratory tract infections) which may be used as targets for potential antimicrobial agents.

AAF94410 to AAF94410 trepresent PCR primers used in the exemplification of the present invention.
Essential bacterial genes from Haemophilus influenzae and methods for identifying 'essential' genes that may be potential therapeutic targets
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              84 ETWRRELAAEFEKPYFKQLMSFVADER-SRHTVYPPADQVYSSTEMCDIQDVKVVILGQD 142
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            203 VLTVRAHQANSHKDRGWETFTDAVIKWLSVNREGVVFLLWGSYAHKKGATIDRKRHHVLQ 262
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          PYHGPNQAHGLCFSVQKPVPPPPSLVNIYKELCTDIDGFKHPGHGDLSGWAKQGVLLLNA 202
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             Length 219;
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                DB 22;
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; Pred. No. 4.6e-57
28; Mismatches 6
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         263 AVHPSPLSAHRGFLGCKHFSKANGLLKLSGTEPINWR 299
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                                                                                                              Page 53; 185pp; English.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          Query Match
Best Local Similarity 57.1%;
Matches 124; Conservative 28
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            219 AA;
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               Sequence
                                                                                                                    Claim
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